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Mutualistic relationships between the shield ant, *Meranoplus bicolor* (Guérin–Méneville) (Hymenoptera: Formicidae) and honeydew–producing hemipterans in guava plantation

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Abstract

Mutualistic relationships between the shield ant, *Meranoplus bicolor* (Guérin–Méneville), and two species of hemipteran, *Aphis gossypii* Glover and *Ferrisia virgata* (Cockerell), were investigated in an unsprayed guava plot at Kamphaeng Saen, Nakhon Pathom, Thailand. The reciprocal benefits were observed in both field and laboratory studies. *M. bicolor* activity coincided with peak seasonal activity of both hemipterans during June–August. We indicated two sets of support evidence in *M. bicolor* honeydew preference: (i) statistically higher value of adjusted honeydew weight collected by ant workers from *A. gossypii* compared with that from *F. virgata* (p -value = .005), and (ii) the higher value of the strength of effect ($\eta^2 = .62$) in the total variance of multi-species association. The physical property on honeydew viscosity was discussed concerning ant preference. We used two–group, ant–tended and ant–excluded, between–subjects multivariate analysis of variance (MANOVA) in order to show hemipteran benefits. Both hemipteran populations increased in the ant–tended treatment, together with lesser amounts of two species of coccinellids, *Menochilus sexmaculatus* (Fabricius) and *Coccinella transversalis* Fabricius, and one species of syrphid fly, *Pseudodorus clavatus* (Fabricius), compared with the ant exclusion treatment (p -value <.001). The facultative mutualistic relationships of *M. bicolor* and the two hemipteran species were mentioned.

Introduction

The shield ant, *Meranoplus bicolor* (Guérin–Méneville) (Hymenoptera: Formicidae), is a common ground nesting species of the subfamily Myrmicinae, and is widely distributed throughout the entire Oriental Region (Schödlh, 1998). The workers not only forage on dead arthropods as scavengers, but also collect honeydew as carbohydrate source from hemipterans, e.g. the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphidae) and the striped mealybug, *Ferrisia virgata* (Cockerell) (Hemiptera: Pseudococcidae), in agricultural ecosystem. However, the trophobiotic relationships or mutualism between *M. bicolor* and honeydew–producing hemipterans are unknown.

Mutualism between ants and honeydew–producing hemipterans has been identified as a continuum of relationships ranging from mutualistic to antagonistic (Stadler & Dixon, 2005; Billick et al., 2007), and hemipterans tending by

ants are mostly facultative or opportunistic (Delabie, 2001). Generally, ants benefit from associations with hemipterans by obtaining carbohydrate–rich food source in the form of “honeydew” secreted from hemipterans (e.g.: Nixon, 1951; Way, 1963; Hölldobler & Wilson, 1990). Specifically, the benefits to ants have been focused on the foraging behavior of worker ants (Stadler & Dixon, 2005; Grover et al., 2007; Kay et al., 2010). Some have concentrated on fitness benefits in terms of ant colony growth (Grover et al., 2007; Helms & Vinson, 2008; Wilder et al., 2011). In return the benefits, ants may reduce hemipteran contamination of their waste products, removing dead individuals, protecting natural enemies, and transport hemipterans to new feeding sites, resulting in the abundance of hemipteran populations (e.g.: Way, 1963; Nielsen et al., 2010; Stadler & Dixon, 2005). Ants exploit hemipterans not only for their honeydew, but also as a protein source when foraging on them as a common prey (Buckley, 1987; Hölldobler & Wilson, 1990; Delabie, 2001). However, this



type of antagonistic relationships will not be treated here; we are looking at a concrete evidence of mutually benefits among both partners.

In this study we verified, in both field and laboratory experiments, the reciprocal benefits of *M. bicolor* and two species of honeydew-producing hemipterans, *A. gossypii* and *F. virgata*. We concentrated for over three-month period in the guava plantation of Horticulture Department, Kasetsart University, Nakhon Pathom, Thailand, observing the mutualism of ant-hemipterans including the abundance of natural enemies, mainly predators. We tested three hypotheses: (i) ants receiving benefits in terms of honeydew from mutualistic associations in guava agroecosystem; (ii) ants protecting hemipterans from natural enemies therefore the densities of natural enemies decrease in the presence of ants; and (iii) in consequence of the two hypotheses mentioned earlier, resulting in the increments of hemipteran densities in ant-hemipteran associations compared with the ant-exclusion arrangement.

Materials & Methods

Study species

The study was conducted during April–December 2012 in the unsprayed varietal collection plots (varieties: Phant Si Thong, Kim Ju, and Vhan Pi Roon), consisting of 336 guava trees, *Psidium guajava*, of Horticulture Department, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Thailand (14.0358 °N, 99.9826 °E). The predominant ground-nesting ant species in the study area was the native *M. bicolor*, with only a few colonies of the invasive ant species the tropical fire-ant, *Solenopsis geminata* (Fabricius) near the perimeter of the plantation. The honeydew-producing hemipterans were *A. gossypii* and *F. virgata*. The natural enemies, mainly predators, were two species of coccinellid beetles, *Menochilus sexmaculatus* (Fabricius) and *Coccinella transversalis* Fabricius (Coleoptera: Coccinellidae), and one species of syrphid fly, *Pseudodorus clavatus* (Fabricius) (Diptera: Syrphidae).

Ant benefit and honeydew preference

The direct benefit of *M. bicolor* was obtained by weighing a certain number of foraging ants, and then calculating the difference of weight gain between foragers descending and ascending the guava branches. We measured weight gains of *M. bicolor* after visiting hemipteran colonies as honeydew receiving. We randomly chose foraging ants from the field to weigh for honeydew loading; 50 ant foragers ascending the guava branch before reaching hemipteran colonies, and the other 50 individuals descending the branch with full load of honeydew. Honeydew loads were measured from the weight differences of ants filled with honeydew and ants ascending the guava branch. Individual worker of *M. bicolor* was cap-

tured in an empty hard gelatin capsule (size 0; outer diameter 7.65 mm, height 21.7 mm, and volume 0.68 ml of Torpac Inc., NJ), and shortly after, the capsule containing the arrested ant was weighed on a digital balance. The actual ant weight was obtained from the subtraction of the capsule weight. We weighed, from field collected, two sets ($n = 200$) foraging ants visiting *A. gossypii*, and one set ($n = 100$) of ants visiting *F. virgata*. The honeydew loads were confirmed with the laboratory set up by feeding of *M. bicolor* workers with honeydew. A set of field collected workers ($n = 100$) leaving their nests for foraging were randomly chosen, holding in captivity for 24 hours without food, and subsequently captured inside the gelatin capsule for weighing. After weighing, half of the 24-hour arrested *M. bicolor* was offered with guava leaves occupied by honeydew exudates of *A. gossypii*, and the other half of ants with honeydew from *F. virgata*. The ants were allowed to feed on honeydew until they either refused to feed or left the guava leave. All *M. bicolor* workers were weighed for the second time in order to obtain honeydew loads before releasing back to their former habitats.

Hemipteran benefits

We randomly selected 30 guava trees, age 6 years old, approximately 1.65 m in height and 2.5–2.75 m in diameter from the pesticide-free guava plot as our study units. One of two similar branches was randomly chosen from each selected tree to perform ant-exclusion treatment, using sticky barrier around the base of the branch covering 20 cm in length. The target branch was first wrapped around with plastic wrap, and then applied with generic horticultural glue (colorless and odorless). The objective of the gluey barrier is to prevent ants and other crawling insects from reaching hemipteran colonies at the guava shoots, allowing only the entering of air-borne insects, including winged aphids, mealybug crawlers, ladybugs, and syrphid flies. The barriers were examined periodically, and reapplied the glue as required, in order to maintain the effectiveness as ant barriers throughout the experimental period. The other branch was left unmanipulated as the ant-presence treatment. There was the total of 60 experimental units. This ant-exclusion/presence experiment was started in May, beginning with equal numbers of both *A. gossypii* and *F. virgata* between the two treatments on the same guava tree. Insect observations were made during peak seasonal activities of both hemipterans and their natural enemies in June–August 2012.

On each chosen guava tree, we randomly selected one terminal shoot from the total of 3–5 shoots of each experimental unit, in order to make observations. All terminal shoot belonging to each experimental unit had an equal chance to be picked on each data collection day. The number of hemipterans: *A. gossypii*, *F. virgata*; and larvae of predators: *M. sexmaculatus*, *C. transversalis*, and *P. clavatus*, occupying the branch terminal side of 30 cm in length of both presence

and absence of *M. bicolor* were counted at various intervals throughout the duration of the experiment from April–December 2012. We counted the insects at interval of 3–5 days, with the total of 7 times per month during June–August, coincided with the peak activities of both hemipterans and their predators, and every 15 days in other months. However, the observation data or multivariate responses of the five dependent variables were derived from the average of 7 times \times 3 months = 21 field observations during peak activities of the insects in June–August 2012. We recorded the number of *M. bicolor* moving up or down (bidirectional) passed a fixed point on the treatment branch with no gluey barrier for 3-min period, to ascertain ant activity throughout the overall experimental period from April–December 2012. All observations in the field were done during 08:30–11:30 hours.

Statistical analyses

To answer the question on the difference of honeydew weights or ant's honeydew preference between *M. bicolor* collecting *A. gossypii* honeydew compared with those of *F. virgata*, we used analysis of covariance (ANCOVA) of IBM SPSS Statistics (Verma, 2013; Meyer et al., 2013). Body weight of *M. bicolor* workers with empty stomach (24-hour unfed workers) or weight before receiving honeydew was treated as covariate, and the criterion variable or dependent variable was ant weight after eating honeydew from each hemipteran species. The analysis of covariance approach was used in order to adjust the initial variations of *M. bicolor* worker size.

The honeydew-producing hemipteran benefits were demonstrated by interference of ants, predominantly *M. bicolor*, with sticky barrier applying around the base of the main branch in order to exclude the ant. The abundance of hemipterans and natural enemies were compared between presence and absence of *M. bicolor*. We anticipated more hemipterans and less natural enemies in the ant-attended guava branches.

Most studies of ant-hemipteran interactions included either ant or hemipteran removals from the study plants, and made comparisons with the unmanipulated partners. The conclusions, in general, relied on statistical analysis by the uses of univariate analysis of variance (ANOVA), which concentrated on one dependent variable, with attempts to make findings from multiple analyses of ANOVA (e.g.: Flatt & Weisser, 2000; Billick et al., 2007; Daane et al., 2007; Mgocheki & Addison, 2009; Styrsky & Eubanks, 2010). Herein we used multivariate analysis of variance (MANOVA) of IBM SPSS Statistics (Meyer et al., 2013; Rencher & Christensen, 2012), in order to draw one solid conclusion of ant-hemipteran mutualism based on the comparison of five dependent variables from two groups, presence and absence of *M. bicolor* on guava branches. These five dependent variables or multivariate responses were number of insects: i.e. nymphs and adults of *A. gossypii*; nymphs and adults of *F. virgata*; larvae of

M. sexmaculatus; larvae of *C. transversalis*; and larvae of *P. clavatus*. All insect counts were transformed into log ($y + 1$) format; where y = number of insect, in order to agree with statistical assumptions.

Several outputs were requested from the MANOVA analysis of IBM SPSS. Box's Test of Equality of Covariance Matrices expected to see if the dependent variable covariance matrices are equal across the levels of the presence-absence of *M. bicolor*. Bartlett's Test of Sphericity was demanded to ascertain sufficient correlation between dependent measures in order to proceed with the analysis. The core MANOVA output was inquired for the multivariate null hypothesis evaluation of no differences between presence and absence of *M. bicolor* on the composite dependent (number of insects) variate. When the multivariate test is statistically significant, we can proceed with some assessments of each dependent variable. We performed the Tests of Between-Subjects Effects to evaluate the statistical significance of each dependent variable separately. Bonferroni-corrected alpha level was applied to avoid alpha inflation in order to evaluate these presence and absence of *M. bicolor* effects. We divided .05 by the number of ANOVAs and obtained .05/5 or a Bonferroni-corrected alpha level of .01.

Results and Discussion

M. bicolor generally foraged on honeydew of hemipterans as carbohydrate source throughout the year in guava plantation at Kamphaeng Saen. Monthly averages (\pm SE) of *M. bicolor* activity from April–December 2012 are presented in Fig 1. *M. bicolor* activity coincided with population fluctuations of both hemipterans (*A. gossypii*, and *F. virgata*), with peaks seasonal activities in June–August (Fig 1). There were very high correlation coefficients (r 's) between ant activity and either *A. gossypii* or *F. virgata* density at $r = .97$ (p -value $< .001$; $n = 9$) and $r = .93$ (p -value $< .001$; $n = 9$), respectively.

M. bicolor dominated the other ground-nesting ant species, *Solenopsis geminata*, in the studied guava plot, although *S. geminata* has been considered as one of the most invasive ant species worldwide (Wetterer, 2011), but not in this guava ecosystem with history of pesticide applications. There were no *S. geminata* workers observed on the experimental guava trees. The tolerance to pesticides of *M. bicolor* was probably due to the protection of long fine hair covering the entire body (Schödlh, 1998), together with the defensive behavior of *Meranoplus* by curling up the body and feigned dead when disturbed (Hölldobler, 1988).

Ant benefit and honeydew preference

In the studied guava plantation, foragers of *M. bicolor* leaving their nests weighed approximately 2.48 mg (SE = .08; $n = 150$). After visiting hemipteran colonies, *M. bicolor* with honeydew loaded, descending the branch back to their nests weighed on average 8.69 mg (SE = 0.1; $n = 150$). The

honeydew loading is about 6.21 mg (8.69 – 2.48) or roughly estimate around 2.5-fold ($6.21 \div 2.48$) of the mean forager weight departure from their nests. The weighting capacity of *M. bicolor* workers was reconfirmed in a confined study of laboratory feeding of ant workers to different kinds of honeydew from both hemipteran species. After 24 hours in captivity, *M. bicolor* workers weighed 3.61 mg (SE = 0.12; n = 100) on average. We selected larger workers with more tolerance and easier for seizing in order to withstand the 24-hour starvation before obtaining honeydew. These workers were fully fed with honeydew from different hemipteran species, and later weighed approximately 10.70 mg (SE = 0.15; n = 100). The overall expected value of honeydew loading is 7.09 mg (10.70 – 3.61), with an estimate of 2.96-fold ($7.09 \div 3.61$) of the average worker weight after 24 hour in caging. The former 2.5-fold honeydew loading from field foragers was slightly lesser; this was probably due to the offering of honeydew by trophallaxis among workers before returning to their nests (Pfeiffer & Linsenmair, 2007).

One-way between-subjects ANCOVA assessing the difference of honeydew loadings from two hemipteran species of *M. bicolor* workers showed that the covariate effect or weight of 24-hour captured *M. bicolor* before honeydew feeding was statistically significant, $F_{(1, 97)} = 786.297$, p -value < .001. Moreover, a statistically significant effect of honeydew source, from either *A. gossypii* or *F. virgata* honeydew, was obtained, $F_{(1, 97)} = 8.387$, p -value = .005.

Mean weight of ants before eating honeydew of *F. virgata* group was higher than that of *A. gossypii* group, leading to higher full up honeydew loading from *F. virgata* compared with that from *A. gossypii* (Fig 2). However, the use of ANCOVA approach removed the covariate effect and unveiled the reversal outcome. Mean weight of *M. bicolor* plus honeydew from *A. gossypii* was significantly higher when corrected for weight prior to receiving honeydew (adjusted mean = 10.85; SE = .072; 95% CI = 10.707–10.992) than mean weight of

worker ant with honeydew loaded from *F. virgata* (adjusted mean = 10.55; SE = .072; 95% CI = 10.408–10.693) (Fig 2). This could indicate that *M. bicolor* workers prefer honeydew from *A. gossypii* to that from *F. virgata*.

Ants are expected to concentrate their honeydew collection activities on hemipteran species offering higher reward in terms of both quantitative and qualitative effects. Hemipteran species that produce larger amount of honeydew, or having honeydew with the presence of preferred sugars or amino acids should be more attractive to certain ant species (Cushman, 1991; Völkl et al., 1999; Yao, 2014). Ant preference for particular sugars in hemipteran honeydew can be species specific (Blüthgen & Fiedler, 2004). Several ant species react strongly to honeydew that holds large amounts of melezitose (Völkl et al., 1999), while others prefer sucrose to melezitose (Blüthgen & Fiedler, 2004). On the other hand, *A. gossypii* honeydew consisted of mainly sucrose, fructose, and erlose (Lawo et al., 2009), with no appearance of melezitose.

Honeydew composition of *F. virgata* is unknown; however, some studies of mealybugs' honeydew show composition of fructose, glucose, sucrose, and small amounts of melezitose and raffinose, together with a variety of amino acids (Gray, 1952; Salama & Rizk, 1969).

Another difference in honeydew quality beside the composition of sugars and amino acids is a physical property specifically honeydew viscosity. In our study, honeydew excreted by *F. virgata* was more viscous than that by *A. gossypii*, which their honeydew seemed to be watery liquid. A study in Argentine ant showed that workers fed eightfold longer on gel sucrose composition, and removed fivefold less sucrose than workers feeding on liquid sucrose (Silverman & Roulston, 2001). The later would agree with lesser amounts of honeydew loading of *M. bicolor* from *F. virgata* than that from *A. gossypii* in this study.

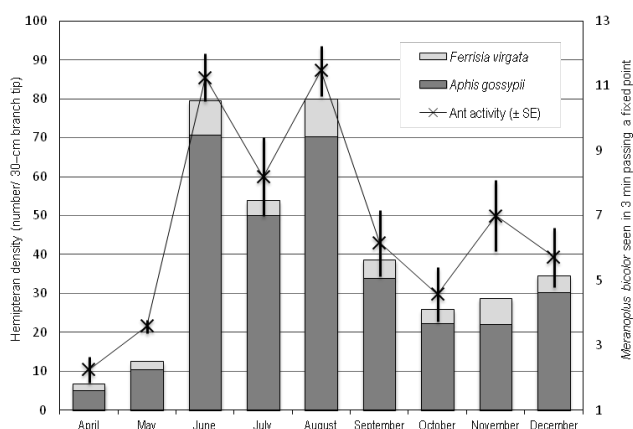


Fig 1. Monthly average of *Meranoplus bicolor* activity (\pm SE, vertical line), and hemipteran densities (*Ferrisia virgata* and *Aphis gossypii*) from 30 ant-tended guava branches at Kamphaeng Saen, Nakhon Pathom, Thailand in year 2012.

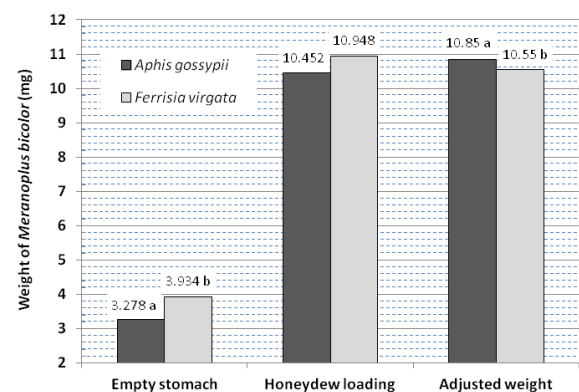


Fig 2. Mean weight of *Meranoplus bicolor* workers (mg) with empty stomach (24-hour without food), mean weight with honeydew loading, and adjusted mean weight from different hemipteran species, *Aphis gossypii* and *Ferrisia virgata*. Value on top of column chart indicates data label; different letters followed mean values represent statistically significant differences (p -values $\leq .005$).

Hemipteran benefits

A two-group between-subjects MANOVA was done on logarithmic transformed data [$\log(y + 1)$; y = observation data] of five dependent variables: no. of *A. gossypii*; no. of *F. virgata*; no. of *M. sexmaculatus* larvae; no. of *C. transversalis* larvae; and no. of *P. clavatus* larvae. The independent variable or treatment was the presence-absence of ants, particularly *M. bicolor*, in guava plantation. There were two treatments, i.e. ant-tended and ant-excluded. In general, the ant-excluded treatment with sticky barrier was quite effective against *M. bicolor*, the slow-moving ant species. Even though some ants could accidentally reach the colonies of hemipterans on the exclusion treatment from adjacent branches due to the contact with nearby branches via wind blowing, however, these ants could not return back to their nests or could not be able to recruit additional ant foragers.

The sample consisted of 60 guava branches divided into equal amounts of presence and absence of *M. bicolor*. The output of Box's Test of Equality of Covariance Matrices was statistically significant (Box's $M = 69.998$; p -value $< .001$), showing that the dependent variable covariance matrices were not equal across the levels of the presence-absence of *M. bicolor*. Therefore, Pillai's trace was used to evaluate all multivariate effects (Meyer et al., 2013). Bartlett's Test of Sphericity was statistically significant (approximate chi-square = 99.838; p -value $< .001$), indicating sufficient correlation between the dependent variables to proceed with the MANOVA. Using Pillai's trace as the criteria, the combined dependent variable was significantly affected by the presence-absence of *M. bicolor*, Pillai's trace = .807, $F_{(5, 54)} = 45.244$, p -value $< .001$. There were reliable multivariate differences between ant-tended and ant-excluded treatments on the combined dependent variate. The partial eta squared = .807 (partial η^2), equivalent to the full eta squared (η^2) in this two-group design (Levine & Hullett, 2002), indicating that we had a very high proportion of the total variance (.807, or about 81%) explained by the activity of *M. bicolor*.

Each dependent measure or each observed insect density was assessed individually in order to determine the strength of the statistically significant multivariate effect. The result of the tests of the univariate effects is shown in Table 1. We had statistically significance univariate effects on all dependent variables (Table 1; p -values $< .001$). Of all insect species under investigation, *A. gossypii* provided the highest effect size ($\eta^2 = .62$), while *M. sexmaculatus* had highest effect size in terms of natural enemies ($\eta^2 = .55$) (Table 1).

The descriptive information for the univariate analysis is presented in Fig 3; providing each dependent measure's observed means, and total averages obtaining from 30 guava trees in the study. The presence of *M. bicolor*-tended hemipterans had a considerable impact on insect populations not only hemipteran themselves, but also their natural enemies. On ant-tended treatment, we detected higher densities of both

hemipteran species, together with lesser amounts of all natural enemies compared with the ant-excluded treatment (Fig 3). There was more abundant in density of roughly 7.6-fold [$\{\text{antilog}(1.824) - 1\} \div \{\text{antilog}(1.002) - 1\}$ or $68.50 \div 9.05 = 7.57$] of *A. gossypii* than *F. virgata* from 30 guava trees in the study (Fig 3).

In general, we would say that *M. bicolor* preferred to associated with *A. gossypii* more than *F. virgata*, in this meaning the preference of honeydew collecting, as indicated by higher value of the strength of effect or effect size (Levine & Hullett, 2002; Meyers et al., 2013), i.e. $\eta^2 = .62$ and $\eta^2 = .52$, respectively (Table 1). This could be the second evidence in supporting the previous study of honeydew preference of 24-hour captured *M. bicolor*. Among the three natural enemies or predators, *M. sexmaculatus* had more strength of effect ($\eta^2 = .55$), i.e. would be more effective predator, than the other two competitors (η^2 s = .39, and .32) in this *M. bicolor*–hemipteran association (Table 1). Even though the surphid fly, *P. clavatus*, was more abundant than the other two coccinellid predators, but its appearance in the guava plot was restrict to June till August. In addition, there were no *P. clavatus* larvae found preying on the striped mealybug, *F. virgata*, in our study.

The mutualistic relationships or trophobiotic interactions between either *A. gossypii* or *F. virgata* with ants have been classified as facultative and very common phenomenon by Delabie (2001). There are two main reasons from this study in supporting the above mentioned: firstly, both hemipteran species are polyphagous and cosmopolitan species (Blackman & Eastop, 2000; da Silva-Torres et al., 2013), any mutualistic relationship with ants should be opportunistic or facultative rather than obligatory; and secondly, *M. bicolor* is the most common species of the genus *Meranoplus* in the Oriental Region (Schödlh, 1998), and is widely distributed as ground nesting species in disturbed habitats of agricultural

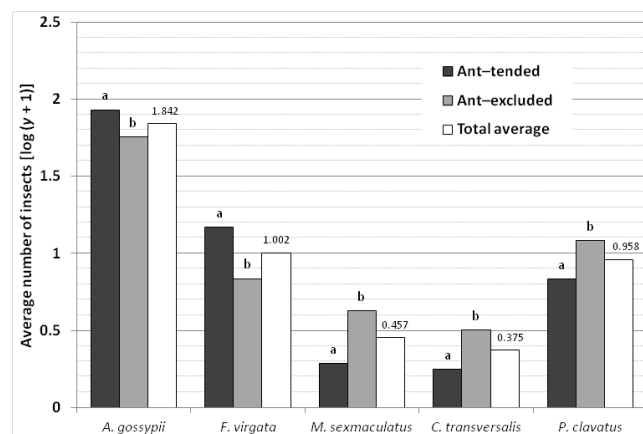


Fig 3. Effect of *Meranoplus bicolor*-exclusion on hemipterans (*Aphis gossypii* and *Ferrisia virgata*) and their natural enemies (*Menochilus sexmaculatus*, *Coccinella transversalis* and *Pseudodorus clavatus*). Different letters on top of dark columns represent statistically significant differences (p -values $< .001$); numbers on top of clear columns indicate data labels of total average or grand mean from 30 guava trees.

Table 1. Tests of univariate effects of *Meranoplus bicolor* activity on five dependent variables^a: *Aphis gossypii*; *Ferrisia virgata*; *Menochilus sexmaculatus*; *Coccinella transversalis*; and *Pseudodorus clavatus*. Data are visual counts of insects occupying 30-cm length of branch terminal (n = 60)

Source	Dependent var.	Type III SS	df	MS	F	p-value	Eta squared (η^2)
Ant	<i>A. gossypii</i>	.466	1	.466	96.188	.000 ^b	.624
	<i>F. virgata</i>	1.681	1	1.681	61.859	.000	.516
	<i>M. sexmaculatus</i>	1.744	1	1.744	72.151	.000	.554
	<i>C. transversalis</i>	.977	1	.977	27.876	.000	.325
	<i>P. clavatus</i>	.925	1	.925	37.525	.000	.393
Error	<i>A. gossypii</i>	.281	58	.005			
	<i>F. virgata</i>	1.576	58	.027			
	<i>M. sexmaculatus</i>	1.402	58	.024			
	<i>C. transversalis</i>	2.034	58	.035			
	<i>P. clavatus</i>	1.430	58	.025			
Corrected total	<i>A. gossypii</i>	.746	59				
	<i>F. virgata</i>	3.257	59				
	<i>M. sexmaculatus</i>	3.146	59				
	<i>C. transversalis</i>	3.011	59				
	<i>P. clavatus</i>	2.355	59				

^a The multivariate test of combined dependent measure was statistically significant; Pillai's trace = .807, $F_{(5, 54)} = 45.244$, p -value < .001.

^b .000 indicates < .001.

ecosystem, therefore the acquiring for food in the vicinity should be by selection of most abundant resources.

This study showed that ant attending had a considerable effect on hemipterous pest densities in guava plantation. There were more individuals of hemipterans in the ant tending guava branches, together with lesser amounts of natural enemies mainly predators because of ant guarding activities. In return the benefit, ants received carbohydrate sources in terms of honeydew from both hemipterans. In general, the results of this ant exclusion experiment using gluey barrier are agreed with previous studies done in fruit orchards; e.g. cherry (Stutz & Schmidt-Entling, 2011), apple (Stewart-Jones et al., 2008; Miñarro et al., 2010; Nagy et al., 2013); and in vineyards (Mgocheki & Addison, 2009).

In conclusion, mutualistic relationships between *M. bicolor* and honeydew-producing hemipterans were revealed. *M. bicolor* preferably collected honeydew of *A. gossypii* more than that of *F. virgata*, because it was not only easier to find, i.e. more abundant, but also more ingestible, due to the physical property of watery liquid. Other alluring properties of honeydew to ants could be honeydew composition in terms of sugars and amino acids, which needed further investigations. The ant-exclusion promoted an increment in predator densities, and thus leading to a tentatively conservation biological control of hemipterous pests in guava agroecosystem.

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